Presence of Simian Virus 40 DNA Sequences in Egyptian Patients with Lymphoproliferative Disorders

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Abstract:

Background: Although no definite risk factors have emerged for the different hematological malignancies, a viral cause has been postulated. Several studies have detected SV40 DNA sequences in tumor tissues obtained from non-Hodgkin's lymphoma patients. A link between SV40 and NHL is biologically plausible because SV40 causes hematological malignancies in laboratory rodents.

Methods: We investigated 266 Egyptian cases of different hematological malignancies, for the presence of SV40 DNA using multiplex nested PCR technique. These cases consisted of 158 non-Hodgkin's lymphoma (NHL), 54 Hodgkin's disease(HD), 26 acute lymphocytic leukemia (ALL), 13 acute myeloid leukemia (AML), 8 chronic lymphoblastic leukemia (CLL), 7 chronic myeloid leukemia (CML), in addition to 34 subjects of control group.

Results: Our results have shown that SV40 DNA sequences were found in 53.8% of non-Hodgkin lymphoma patients, 29.6% of Hodgkin's disease patients, and 40.7% of different types of leukemia cases. Frequency of SV40 DNA sequences was higher in NHL patients compared to the other tumor cases. Also, frequency of SV40 DNA sequences was significantly higher ($p<0.05$) in NHL patients than in the control group. Regarding the different histological types of non-Hodgkin's lymphoma, SV40 DNA sequences were detected frequently in diffuse large B-cell lymphoma and in follicular lymphoma.

Conclusions: The present study suggests that SV40 DNA virus is significantly associated with non-Hodgkin's lymphoma and might have a role in the development of these hematological malignancies. Polyomavirus SV40 may act as a cofactor in the pathogenesis of these tumors and this could lead to new diagnostic, therapeutic, and preventive approaches.

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Introduction

SV40 is a member of polyomaviruses with icosahedral capsids containing small, circular, double-stranded DNA genomes. SV40 has been reported to transfer to humans by contaminated poliovirus vaccines that were widely used in Western countries before 1963.[1] Several studies have detected the DNA of Simian virus 40 (SV40) in tumor tissues obtained from non-Hodgkin's lymphoma (NHL) patients.[2-4] A link between SV40 and NHL is biologically plausible because SV40 causes leukemia and lymphoma in laboratory rodents.[5-7]

Transformation of rodent and human cells by SV40 is induced by the two oncoproteins encoded in the early region of the viral genome, the large tumor antigen (Tag), and the small tumor antigen (tag).[8] Laboratories that have used sensitive polymerase chain reaction (PCR) methods to detect SV40 DNA in NHL tumor tissue have described variable results. Four U.S. studies reported detecting SV40 T antigen sequences in tumor tissue from 15–43 percent of NHL patients.[2,3,9,10] SV40 T antigen sequences were also found in NHL tissues from 11 percent of Japanese patients.[4] Additionally, SV40 sequences were detected in 14 percent of NHL specimens from Italy.[11] On the other hand, two other European studies of patients with lymphoproliferative disorders showed strikingly negative results.[12,13] In a study based in Italy and Spain[12], SV40 T antigen sequences were found in only 3 percent of tissues using 2 sets of PCR primers, and none of the initially positive specimens was positive using a third, confirmatory primer set. In a study from the United Kingdom.[13] SV40 T antigen sequences were not detected in any NHL specimens using quantitative real-time PCR, despite a detection limit of 10 SV40 copies in 100 ng genomic DNA (i.e., approximately one SV40 copy per 1,500 cells). The reasons for this variability in results from PCR-based studies are unclear. Although varying results from different countries might reflect geographic differences in the patient populations, the results varied somewhat even among the studies conducted in the U.S.[2,3,9,10] and the Italian.[11,12] Differences in PCR testing conditions, such as the selection of PCR primers or reaction settings, might be contributing to the variability in results.

Although these observations indicate an association between SV40 and non-Hodgkin's lymphoma, they do not demonstrate a causal role. In Egypt, nearly all children were not exposed to contaminated polio vaccine prior to 1963. However, the relationship between SV40 infection and different hematological malignancies is unclear and needs further studies. Hence, the purpose of this study was to investigate SV40 DNA sequences in these Egyptian patients, where immunocompromised individuals are known to be at risk of development of virus-mediated neoplasm [14] and determine the incidence of SV40 DNA positivity in different hematological malignancies.

Methods

Clinical Specimens: Fresh tumor samples from 212 Egyptian patients with lymphoma and 54 blood samples of different types of leukemia in addition to 34 controls were collected from National Cancer Institute hospital, Cairo University, Egypt, during the period from February, 2004 to December, 2005. 158 patients were of non-hodgkin's lymphoma (NHL), 54 patients were of Hodkin's disease (HD), and 54 patients were of different types of leukemias (26ALL, 13AML, 8CLL, 7CML).

Cell lines: The HEK293T cell line expressing SV40 *Tag* was used as a positive control for the PCR to detect SV40 genome. On the other hand, HEK293 (JCRB9068) and HEK293JCT cell lines that express *Tag* of JC virus (JCV) were used as negative controls. As well as, plasmid PSVSPH21-N clone as a gift from Dr.Janet Povttz (Baylor College of Medicine, Molecular Virology and Microbiology, Houston, Texas 7703) was used as a control for PCR.

DNA extraction: Extraction of DNA was done according to standard protocols.[15]

Detection of SV40 DNA: SV40 detection was carried out in a multiplex nested PCR technique with the primer sequences and PCR conditions of Fedele et al., 1999.[16] Briefly, 200 ng of the extracted DNA from each sample were amplified in a total of 50 µl of PCR mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl_2, 200 µM of each deoxynucleoside triphosphate, 20 pmol of the outer primers (PM1+ and PM1-) and 2.5 U Taq polymerase. After an initial denaturation step of 2 min. at 94^{o}C for 30 sec., 61^{o}C for 1 min., and 72^{o}C for 30 seconds were done and

a final extension step at 72°C for 5 minutes was performed. A second amplification reaction was carried out by adding 1 µl of the first PCR products to 49 µl of a new reaction mixture comprising 10mM Tris-HCl, 50mM KCl, 4 mM $MgCl_2$, 200µM of each deoxynucleoside triphosphate, 1.25 U Taq polymerase and 20 pmol of the inner primers (SV+ and PM2-). Amplification was carried out under the same conditions used for the first amplification round, except that the annealing temperature was 55°C. A total of 30 cycles were used to complete the second amplification. Amplification products (135 bp for SV40) were analyzed in 3% agarose gel and visualized under UV light (Figure 1).

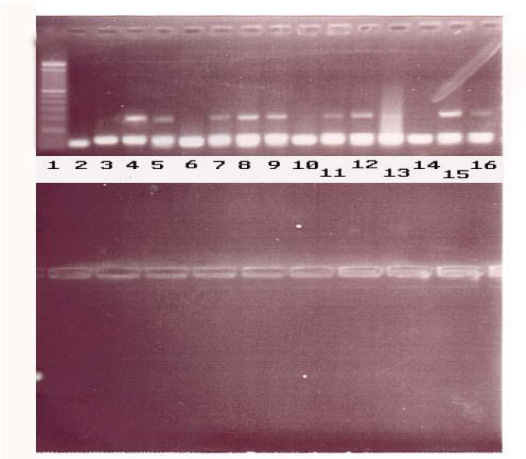

Fig (1). PCR products of SV40 DNA detected by ethidium bromide agarose gel electrophoresis. Lanes 2, 14 represent negative controls which are HEK293 (JCRB9068) HEK293JCT cell lines that express *Tag* of JC virus. Lane15 represents positive control, which is HEK293T cell line expressing SV40 Tag. Lanes 4, 5, 7,8,9,11,12 are positive tumor samples. Lanes 3,6,10 are negative samples.

Statistical Analysis

Statistical analysis was done using SPSS program version 11 statistical software package.Chi-square analyses was used to compare the distribution of viral sequences in non-Hodgkin lymphoma, Hodgkin's disease, and different types of leukemia. Also, t-test was used to compare the mean age of patients with SV40-positive and SV40-negative in the different groups.

Results

This study has been done on 266 Egyptian patients of different hematological malignancies for the presence of SV40 DNA sequences in these cases, in addition to 34 subjects in the control group. One hundred and seventy-eight of cases (59.3%) were males and 122 (40.7%) were females, the age ranged from 2 to 82 years (mean=39.68±18.94). There was no significant difference in the mean age of patients with SV40-positive and SV40-negative non-Hodgkin lymphoma group, and the similar results were observed also in the other groups.

SV40 *Tag* DNA sequences were found in 123 cases out of 266 (46.2%) patients with different hematological malignancies, and 4/34 (11.7%) of control group.

Table (1) shows that there was a statistically significant difference between the presence of SV40 in non-Hodgkin lymphoma cases and in other cases (p=0.000). Also, Frequency of SV40 DNA sequences was significantly higher (p<0.05) in NHL patients than in the control group.

Table (1). SV40 DNA in different hematological malignancies

Group	SV40 DNA sequences (PCR)	
	Negative	**Positive**
NHL	73/143 (51%)	85/123 (69.1%)
HD	38/143 (26.5%)	16/123 (13%)
Leukemias	32/143 (22.3%)	22/123 (17.9%)
Total	143	123
Controls	30 (88.2%)	4(11.8%)

Chi-square = 25.1; p = 0.000

Eighty-five patients out of 123 (69.1%) SV40-positive cases were of non-Hodgkin's lymphoma (NHL), and 16/123(13%) cases were of Hodgkin's disease (HD). On the other hand, 22/123(17.9%) cases of different types of leukemia were of SV40 positive. In the 34 control group (11 bone marrows + 23 blood donors), SV40 were positive in 4 individuals (one bone marrow and three blood donors).

Fifty-three percent of non-Hodgkin lymphoma patients were positive for SV40 sequences and (29.6%) of Hodgkin's disease patients were SV40 positive, while (40.7%) of different types of leukemia cases were SV40 positive. Also, the frequency of SV40 infection in the different histological types of non-Hodgkin lymphoma is summarized in Table (2).

Table (2). SV40 DNA in different types of leukemias and Non-Hodgin's lymphoma

Group	Type	Number positive/number tested	Percentage
NHL	Total	85/158	53.8%
	Diffuse large B-cell	31/51	60.7%
	Follicular lymphoma	19/35	54.2%
	Mantle cell lymphoma	6/13	46.1%
	Burkitt's lymphoma	14/18	77.7%
	MALT lymphoma	6/18	33.3%
	Anaplastic large T-cell	9/23	39.1%
HD		16/54	29.6%
Leukemias	Total	22/54	40.7%
	ALL	11/26	42.3%
	AML	6/13	46.1%
	CLL	3/8	37.5%
	CML	2/7	28.5%
Controls	Total	4/34	11.7%
	Blood donors	3/23	13%
	Bone marrows	1/11	9%

Chi-square = 36.4; p = 0.000

In order to confirm the presence of SV40 T antigen sequences, we further analyzed samples of non-Hodgkin lymphoma in which SV40 DNA was detected. Sequence analysis of amplified products obtained from ten specimens showed the DNA sequences to be identical to those of the SV40 T antigen gene. The sequences associated with NHL lacked a 9 bp insert found in both JC virus and BK virus, proving that the sequences were not derived from either of these polyomaviruses.

Discussion

During the past 30 years, the reported incidence and death rate of the haematological malignancies, with clinical courses ranging from indolent to highly aggressive, have increased strikingly nearly doubling since 1970. No obvious risk factors have emerged for the different haematological malignancies, but a viral cause has been postulated.[14] SV40 DNA sequences have been found in some brain and bone cancers and mesotheliomas.[6,17-19] Polyomaviruses typically establish subclinical and persisting infections in their host, with persistence or latency in several organs, including kidney, brain, and spleen.[14,18] A link between SV40 and NHL is biologically plausible because SV40 causes leukemia and lymphoma in laboratory rodents. Studies have identified SV40, JC, and BK virus DNA sequences in B lymphocytes from HIV-1-infected and HIV-1-uninfected. [7,10,20] However, SV40 has not been shown to be capable of infecting human lymphocytes.[20]

In the present study 53.8% of NHL patients were positive for SV40 sequences and 29.6% of HD patients were SV40 positive, while 40.7% of different types of leukemia cases were SV40 positive. Frequency of SV40 DNA sequences was higher in NHL patients compared to the other tumor cases. This finding is similar to earlier reports.[2,3,21] Presence of SV40 DNA sequences in a large subset of NHL cases suggests that the virus may function as a cofactor in the pathogenesis of this important human tumor. On the other hand, the frequency of SV40 DNA in NHL cases of our results is higher than that reported in previous studies.[4,9-11,21]

This might be a consequence of characteristics of the different populations of patients from whom samples were obtained or the histological types of tumors tested were different. Moreover, this difference could be obtained due to the variations in DNA extraction methods or PCR assay conditions.

Frequency of SV40 DNA sequences was significantly higher ($p<0.05$) in NHL patients than in the control group. This observation coincides with previous studies [2,3], where it was that there was a statistically significant difference between the frequency of SV40 in NHL patients and the control group. This finding suggests that SV40 might contribute to the development of those lymphomas in which it is present.

Regarding the different histological types of non-Hodgkin's lymphoma, SV40 DNA sequences were detected frequently in diffuse large B-cell lymphoma and in follicular lymphoma. This particular association might be important, since these are the two most common histological types of lymphomas from mature B cells and account for about 50-60% of all cases of NHL.[22,23] It also suggests that mature B cells could be more susceptible than precursor cells to the transforming potential of SV40.

The present study suggests that SV40 DNA virus is significantly associated with non-Hodgkin's lymphoma and might have a role in the development of these hematological malignancies. Polyomavirus SV40 may act as a cofactor in the pathogenesis of these tumors and this could lead to new diagnostic, therapeutic, and preventive approaches. Several research approaches seem most likely to lead to new insights that could resolve lingering uncertainly regarding the role of SV40 in human cancer.

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